Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/005169

International filing date: 18 February 2005 (18.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/546,302
Filing date: 20 February 2004 (20.02.2004)

Date of receipt at the International Bureau: 22 July 2005 (22.07.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1344260

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 12, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/546,302*
FILING DATE: *February 20, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/05169*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 962205587 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Kevin J.	WILLIAMS	Wynnewood, PA 19096

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

ANGIOCIDIN FRAGMENTS AND USES THEREOF IN CLINICAL ASSAYS FOR CANCER

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number: 03000

OR

☐ Firm or Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 17 ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☑ Other (specify) Return Postcard

☑ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

☑ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-0075

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($)
SEE FEE TRANSMITTAL FOR FY 2004

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Allan H. Fried

Date February 20, 2004

TYPED or PRINTED NAME Allan H. Fried

REGISTRATION NO. 31,253 *(if appropriate)*

Docket Number: W1107/20007

TELEPHONE 215-567-2010

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

I hereby certify that the above-identified documents are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 with the Express Mail No. and the date indicated above and are addressed to Mail Stop PROVISIONAL PATENT APPLICATION, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Allan H. Fried

Allan H. Fried

EXPRESS MAIL NO. **EV447225648US**

FEE TRANSMITTAL for FY 2004

Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27.

TOTAL AMOUNT OF PAYMENT ($) **80**

Complete if Known	
Application Number	
Filing Date	**February 20, 2004**
First Named Inventor	**James D. Henry**
Examiner Name	
Art Unit	
Attorney Docket No.	**440073.401P1**

METHOD OF PAYMENT

☒ Payment Enclosed:
☒ Check ☐ Credit card ☐ Money Order ☐ Other

☐ Deposit Account:

Deposit Account Number: **19-1090**

Deposit Account Name: **Seed Intellectual Property Law Group PLLC**

The Director is authorized to *(check all that apply)*

☐ Charge fee(s) indicated below ☒ Credit any overpayments
☐ Charge any additional fee(s) during the pendency of this application
☐ Charge fee(s) indicated below, **except for the filing fee**
☒ Charge any deficiencies

to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee($)	Small Entity Fee Code	Small Entity Fee($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	**80**

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		-** =		*		=	
Independent Claims		-** =		*		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** *Reissue independent claims over original patent*
1205	18	2205	9	** *Reissue claims in excess of 20 and over original patent*

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2520	1812	2520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1840*	1805	1840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1480	2254	740	Extension for reply within fourth month	
1255	2010	2255	1005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1510	1451	1510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1330	2453	665	Petition to revive – unintentional	
1501	1330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify) ____					

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY

Name (Print/Type)	**E. Russell Tarleton**	Registration No. Attorney/Agent	**31,800**
Signature	E. Russell Tarleton	Date	February 20, 2004

Customer Number: 00500

ANGIOCIDIN FRAGMENTS AND USES THEREOF IN CLINICAL ASSAYS FOR CANCER

BACKGROUND OF THE INVENTION

1. FIELD OF INVENTION

The present invention relates to assays for detecting the presence of one or more angiocidin fragments as a diagnostic test for cancers and other diseases, the use of such fragments and/or derivatives thereof as calibrators, competitors, and/or indicators in an assay, and to the fragments themselves.

2. DESCRIPTION OF RELATED ART

Cancer is a cellular malignancy which causes the loss of normal control mechanisms and results in unregulated growth, lack of differentiation, and the ability to invade local tissues and metastasize. Thus, cancer cells are unlike normal cells, and are potentially identifiable not only by their phenotypic traits, but also by their biochemical and molecular biological characteristics and by biochemical and molecular biological changes they can induce in non-cancerous tissue, such as nearby stroma. In particular, the altered phenotype of cancer cells indicates altered gene activity, which may be either unusual gene expression, or gene regulation. Identification of gene expression products or proteins, or protein fragments associated with cancer cells will allow for the molecular identification and characterization of malignancies. The ability to diagnose suspected cancers, and to potentially identify not only cell type, but also predisposition for metastasis and any sensitivity to particular anti-cancer therapy, is useful for determining not only the course of treatment, but also the likelihood of success. Uses include, but are not limited to, screening an individual or group of individuals for the presence or development of a cancer; and following a patient with a known cancer for the response to or the effectiveness of therapy, including, for example, a recurrence.

Angiocidin, a cellular receptor, is expressed in invasive tumors (International PCT application, WO 01/05968). Noninvasive tumors either do not express this receptor, or express it at very low levels (WO 01/05968).

The present invention is an assay for fragments of angiocidin, rather than angiocidin itself. The assay is for fragments that are present in the blood, blood plasma, blood serum,

and/or another bodily fluid or sample, such that an elevated concentration of a fragment is taken as an indication that a mammal, particularly a human, has a cancer, a progression of a cancer, and/or a recurrence of a cancer. This invention provides a basis for novel diagnostic assays that can be more specific, more sensitive and/or more easily calibrated, than an assay based on angiocidin itself; i.e., on full-length angiocidin.

All references cited herein are incorporated herein by reference in their entireties.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the detection of angiocidin fragments in the blood, plasma, serum, and/or another bodily fluid or sample from a patient for the purpose of a cancer diagnostic assay.

Angiocidin is a CSVTCG-specific tumor cell adhesion receptor, *see* patent application WO 0105968, also NCBI protein accession number CAC32386.1 and/or CAC32387.1 (corresponding to nucleotide accession numbers AX077201 and AX077202), that in humans has the following amino acid sequences, 380 and 377 amino acids in length, respectively:

CAC32386.1

mvlestmvcvdnseymrngdflptrlqaqqdavnivchsktrsnpennvglitlandcev
lttltpdtgrilsklhtvqpkgkitfctgirvahlalkhrqgknhkmriiafvgspvedn
ekdlvklakrlkkekvnvdiinfgeeevntekltafvntlngkdgtgshlvtvppgpsla
dalisspilageggamlglgasdfefgvdpsadpelalalrvsmeeqrqrqeeearraaa
asaaeagiattgtegerdsddallkmtisqqefgrtglpdlssmteeeqiayamqmslqg
aefgqaesadidassamdtsepakeeddydvxqdpeflqsvlenlpgvdpnneairnamg
slasqatkdgkkdkkeedkk (SEQ ID NO:1)

CAC32387.1

mvlestmvcvdnseymrngdflptrlqaqqdavnivchsktrsnpennvglitlandcev
lttltpdtgrilsklhtvqpkgkitfctgirvahlalkhrqgknhkmriiafvgspvedn
ekdlvklakrlkkekvnvdiinfgeeevntekltafvntlngkdgtgshlvtvppgpsla
dalisspilageggamlglgasdfefgvdpsadpelalalrvsmeeqrqrqeeearraaa
asaaeagiattgtedsddallkmtisqqefgrtglpdlssmteeeqiayamqmslqgaef
gqaesadidassamdtsepakeeddydvxqdpeflqsvlenlpgvdpnneairnamgsla
sqatkdgkkdkkeedkk (SEQ ID NO:2)

For all inventions specified herein, reference to angiocidin is intended to encompass both the form with the amino acid sequence, SEQ ID NO:1, and that with the amino acid sequence, SEQ ID NO:2. Minor variations in the sequence, those involving less than 3% of the amino acids, are also considered to be angiocidin for purposes of the present invention. Such sequence variations are to be distinguished from fragmentation, which implies complete elimination and/or separation of sequences at the amino and/or carboxyl terminus

of angiocidin.

Angiocidin may be derived from cancer tissues, such as melanoma cells or lung carcinoma cells. Analysis by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of mammalian cells has shown that angiocidin has an apparent molecular weight of 50 kD under non-reducing conditions (*See* WO 01/05968). In some of those preparations, small amounts of dimers have been observed with molecular weights of greater than 100 kD (WO 01/05968). Under reducing conditions, the immunoreactive material migrates as two major polypeptide bands spaced closely together with apparent molecular weights of 50 and 60 kD, where the 50 kD species may be a modified form of the 60 kD species or a fragment from degradation (WO 01/05968). This result is consistent with the interpretation that the material consists of two interchain disulfide-linked polypeptide chains that assume a more compact configuration when disulfide bonded. Angiocidin is classified as a glycoprotein since purified immunoreactive material binds galactose-, mannose-, and glucosamine-specific lectins. It does not cross react with antibodies against integrins, laminin, or CD36 (International PCT application, WO 01/05968).

This invention contemplates the use of angiocidin fragments as a method of detecting, diagnosing, and/or following the course of a cancer. Specifically, levels (*e.g.*, concentrations or amounts) of angiocidin fragments can be measured in an organism's blood, blood plasma, serum, biopsy, or other tissue or fluid. In a preferred embodiment, the organism is a mammal, including but not limited to a domesticated animal, pet, a companion animal, a porcine, equine, canine, feline, bovine or mouse. In a most preferred embodiment, the mammal is human. Noninvasive tumors typically do not express angiocidin, or express it only at low levels, whereas invasive tumors express it at high levels. Angiocidin expressed in the context of a tumor is often modified in a way that causes fragmentation, including but not limited to cleavage to separate a sequence segment before secretion by a cell, cleavage after secretion but before exit

from a tumor cell (such as by local proteases), cleavage after exit from the tumor, cleavage in the bloodsteam, and cleavage during sample collection or storage (such as by coagulation proteases that are activated when a blood sample clots during the preparation of blood serum). Said cleavage can be selected from the list consisting of cleavage of an N-terminal sequence, cleavage of a C-terminal sequence, cleavage of a signal peptide, removal of a signal peptide, cleavage of a targeting sequence, removal of a targeting sequence, cleavage of a region that mediates membrane association, cleavage of a region that mediates cell association, removal of a region that mediates association with a membrane or a cell, a proteolytic cleavage, a non-proteolytic cleavage, a hydrolytic cleavage, and an oxidative cleavage. Also contemplated is a fragment or fragments generated as incomplete sequences (illustrative mechanisms include, but are not limited to, alternative splicing and/or mRNA editing). Also contemplated is a combination or combinations of processes to generate a fragment (an illustrative but not limiting example would be alternative splicing to generate an incomplete protein, followed by cleavage to remove a signal peptide sequence). It is understood that secretion versus release via other processes may generate different patterns of angiocidin fragments. As an illustrative but not restrictive example, I conclude that immunoreactive material that is secreted from a cell should be missing a signal peptide and/or a cell-anchoring sequence. Immunoreactive material that is released from a cell via cell lysis will not necessarily have a signal peptide or a cell-anchoring sequence removed. Cell lysis may subject angiocidin or its fragments to lytic processes other than the specific removal of a signal peptide. Therefore, the level of an angiocidin fragment or fragments will be useful in indicating the patient's diagnosis or prognosis. Assay methods include but are not limited to those well-known in the art, such as ELISA, RIA, Western blotting, immunohistochemistry, immunofluorescence, other immune-based methods, non-immune-based methods, quantitative methods, high through-put methods, automated methods,

semi-quantitative methods and qualitative methods.

A kit for the determination of the presence of, and/or the amount of, and/or the concentration of, one or more angiocidin fragments in a material taken or gathered from an organism is also contemplated. A method for generating a binding agent to an angiocidin fragment is also contemplated, in which said binding agent is selected from the group consisting of an antibody, a non-antibody, an aptamer, a binding agent that recognizes an angiocidin fragment, a binding agent that recognizes an angiocidin fragment and angiocidin, and a binding agent that distinguishes between angiocidin fragments and/or between angiocidin and an angiocidin fragment.

While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of diagnosing, following the course, and/or determining the prognosis of a mammal with a cancer said method comprising determining a level of an angiocidin fragment.

2. A method of claim 1 further comprising comparing the level of said angiocidin fragment against known values of the level of said angiocidin fragment determined for one or more members of the same mammalian species that have a metastatic tumor, a nonmetastatic tumor, and/or no cancer.

3. A method of Claim 1 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 376 amino acid residues in length.

4. A method of Claim 3 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 373 amino acid residues in length.

5. A method of Claim 1 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 340 amino acid residues in length.

6. A method of claim 5 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 300 amino acid residues in length.

7. A method of claim 6 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 250 amino acid residues in length.

8. A method of claim 7 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 200 amino acid residues in length.

9. A method of claim 8 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 150 amino acid residues in length.

10. A method of claim 9 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 100 amino acid residues in length.

11. A method of claim 10 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 50 amino acid residues in length.

12. A method of claim 11 wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 25 amino acid residues in length.

13. A method of claim 1 wherein the level of the angiocidin fragment is determined from a sample of bodily fluid.

14. A method of claim 13 wherein the bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an apirate, and cyst fluid.

15. A method of claim 1 wherein the level of angiocidin fragment is determined from a biopsy or other tissue sample.

16. A method of Claim 15 wherein the biopsy comprises a method selected from the group of immunohistochemical staining, immunofluorescent staining, immune staining, nonimmune staining, assay of an homogenate, a quantitative assay, and a quantitative assay normalized to a benchmark.

17. A method of Claim 16 wherein the quantitative assay normalized to a benchmark compises measurement of total protein content.

18. A method of Claim 16 wherein the quantitative assay normalized to a benchmark compises measurement of the total amount of a housekeeping molecule.

19. A method of Claim 18 wherein the housekeeping molecule is actin.

20. A method of claim 1 wherein the mammal is a human.

21. A method that distinguishes angiocidin from an angiocidin fragment, said method

comprising the steps of:

(1) utilizing an epitope or binding target shared by angiocidin and the angiocidin fragment as a target for a binding agent to obtain a quantitation of a total angiocidin plus the angiocidin fragment;

(2) utilizing an epitope or binding target present in angiocidin but not present in the fragment to obtain a quantitation of angiocidin only; and

(3) utilizing the difference between the quantitations obtained in steps (1) and (2) as a quantitation of the amount of angiocidin fragment.

22. A method of Claim 21 wherein the binding agent is an antibody.

23. The method of claim 21 wherein in step (1) and/or step (2) the measurement of an epitope or binding target comprises the use of a binding agent.

24. The method of claim 23 wherein said binding agent comprises a protein and/or a polypeptide.

25. The method of claim 24 wherein said protein comprises an antibody.

26. The method of claim 25 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

27. The method of claim 24 wherein said protein comprises an antibody fragment.

28 The method of claim 24 wherein said protein comprises a single chain antibody.

29. The method of claim 23 wherein said binding agent comprises a non-protein.

30. The method of claim 24 wherein said protein and/or polypeptide is derived from a phage display library.

31. The method of claim 24 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

32. The method of claim 24 wherein said binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

33. A method of claim 24 wherein said binding agent comprises a ligand that binds angiocidin.

34. A method of claim 33 wherein said ligand is selected from the group consisting of a thrombospondin, a thrombospondin fragment that binds angiocidin, a molecule comprising a thrombospondin fragment sequence that binds angiocidin, and a molecule comprising the amino acid sequence CSVTCG (SEQ ID NO:3).

35. A method that distinguishes two angiocidin fragments from each other, said fragments being a first fragment and a second fragment, respectively, said method comprising the steps of:

(1) utilizing an epitope or binding target shared by said first fragment and said second fragment as a target for a binding agent to obtain a quantitation of a total of said first fragment plus said second fragment;

(2) utilizing an epitope or binding target present in said first fragment but not present in said said second fragment, to obtain a quantitation of said first fragment only; and

(3) utilizing the difference between the quantitations in steps (1) and (2) as a quantitation of the amount of said second fragment.

36. A method that distinguishes angiocidin from an angiocidin fragment, said method comprising the step of:

(a) utilizing a binding agent that recognizes an angiocidin fragment but not angiocidin.

37. A method of Claim 36 wherein the binding agent is an antibody.

38. The method of claim 36 wherein said binding agent comprises a protein and/or a

polypeptide.

39. The method of claim 38 wherein said protein comprises an antibody.

40. The method of claim 39 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

41. The method of claim 38 wherein said protein comprises an antibody fragment.

42. The method of claim 39 wherein said protein comprises a single chain antibody.

43. The method of claim 36 wherein said binding agent comprises a non-protein.

44. The method of claim 38 wherein said protein and/or polypeptide is derived from a phage display library.

45. The method of claim 38 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

46. The method of claim 36 wherein said binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

47. A method of claim 46 wherein said binding agent comprises a ligand that binds angiocidin.

48. A method of claim 47 wherein said ligand is selected from the group consisting of a thrombospondin, a thrombospondin fragment that binds angiocidin, a molecule comprising a thrombospondin fragment sequence that binds angiocidin, and a molecule comprising the amino acid sequence CSVTCG (SEQ ID NO:3).

49. A method to detect the presence and/or clinical course of a neoplastic disease by assaying a bodily fluid from an individual, wherein the method comprises the steps of:

(1) measuring the individual's bodily fluid level of an angiocidin fragment or fragments;

(2) utilizing the result of step (1) in a diagnosis as to whether the individual has a neoplastic disease and/or whether a known neoplastic disease has progressed, regressed, or remained stable.

50. The method of claim 49 wherein the bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an aspirate, and cyst fluid.

51. The method of claim 49 wherein the individual referred to therein is a first individual and wherein the method further comprises the steps of:

(3) measuring a second individual's level of the angiocidin fragment in the same type of bodily fluid utilized for step (1), said second individual considered to not have neoplastic disease;

(4) utilizing the result of step (3) in the diagnosis of whether the first individual has a neoplastic disease.

52. The method of claim 49 wherein the first individual's angiocidin fragment level exceeds the angiocidin fragment level of the second individual, and this difference is used to conclude that it is more likely that the diagnosis will be that the first individual has a neoplastic disease and/or a neoplastic disease more advanced than that of the second individual.

53 The method of claim 49, further comprising the steps of assaying the individual's bodily fluid level for an angiocidin fragment more than once, and considering utilizing a change in bodily fluid level from an older to a more recent value to indicate appearance or progression or improvement, wherein said appearance or progression is indicated by an increase in the level of said angiocidin fragment and said improvement is indicated by a decrease in said level.

54. The method of claim 51 wherein the bodily fluid level of an angiocidin fragment is

assayed on 2 or more different days.

55. The method of claim 51 wherein the bodily fluid level of an angiocidin fragment is assayed on more different days 3 spaced at regular intervals, said intervals ranging from two weeks to ten years.

56. The method of claim 49 wherein the neoplastic disease is selected from the group consisting of an adenoma, an adenocarcinoma, a carcinoma, a lymphoma, a leukemia, and a sarcoma.

57. The method of claim 49 wherein the neoplastic disease is an internal cancer.

58. The method of claim 49 wherein the neoplastic disease is selected from the group consisting of a cancer of the respiratory system, a cancer of the circulatory system, a cancer of the musculoskeletal system, a cancer of a muscle, a cancer of a bone, a cancer of a joint, a cancer of a tendon and/or ligament, a cancer of a connective tissue, a cancer of the digestive system, a cancer of the liver and/or biliary system, a cancer of the pancreas, a cancer of the head, a cancer of the neck, a cancer of the endocrine system, a cancer of the reproductive system, a cancer of the male reproductive system, a cancer of the female reproductive system, a cancer of the genitourinary system, a cancer of a kidney, a cancer of the urinary tract, a skin cancer, a cancer of another sensory organ, a cancer of the nervous system, a cancer of a lymphoid organ, a blood cancer, a cancer of a gland, a cancer of a mammary gland, a cancer of a prostate gland, a cancer of endometrial tissue, a cancer of mesodermal tissue, a cancer of ectodermal tissue, cancer of an endodermal tissue, a teratoma, a poorly-differentiated cancer, a well-differentiated cancer, and a moderately differentiated cancer.

59. The method of claim 49 wherein the neoplastic disease is selected from the group consisting of a cancer of solid tissue, a cancer of the blood or the lymphatic system, a solid cancer, a liquid cancer, a non-metastatic cancer, a premetastic cancer, a metastatic cancer, a cancer with vascular invasion, a skin cancer, a poorly differentiated cancer, a well-differentiated cancer and a moderately differentiated cancer.

60. The method of claim 49 wherein the measurement of an angiocidin fragment level

comprises the use of a binding agent, said binding agent capable of binding said fragment.

61. The method of claim 60 wherein said binding agent comprises a protein and/or a polypeptide.

62. The method of claim 61 wherein said protein comprises an antibody.

63. The method of claim 62 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

64. The method of claim 62 wherein said protein comprises an antibody fragment.

65. The method of claim 64 wherein said protein comprises a single chain antibody.

66. The method of claim 60 wherein said binding agent comprises a non-protein.

67. The method of claim 61 wherein said protein and/or polypeptide is derived from a phage display library.

68. The method of claim 61 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

69. The method of claim 60 wherein said binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

70. A method of claim 60 wherein said binding agent comprises a ligand that binds angiocidin.

71. A method of claim 70 wherein said ligand is selected from the group consisting of a thrombospondin, a thrombospondin fragment that binds angiocidin, a molecule comprising a

thrombospondin fragment sequence that binds angiocidin, and a molecule comprising the amino acid sequence CSVTCG (SEQ ID NO:3).

72. The method of claim 49 wherein the angiocidin fragment is separated from angiocidin before said fragment is bound to the binding agent.

73. The method of claim 49 wherein said method comprises the use of a first binding agent, said first binding agent capable of binding angiocidin but not the angiocidin fragment and further comprises a second binding agent, said binding agent capable of binding angiocidin and capable of binding the angiocidin fragment.

74. The method of claim 73 wherein said first binding agent and/or said second binding agent comprises a protein and/or polypeptide.

75. The method of claim 74 wherein said protein comprises an antibody.

76. The method of claim 75 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

77. The method of claim 74 wherein said protein comprises an antibody fragment.

78. The method of claim 74 wherein said protein and/or polypeptide is derived from a phage display library.

79. The method of claim 74 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

80. The method of claim 73 wherein said first and second binding agents are each selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

81. The method of claim 74 wherein said protein comprises a single chain antibody.

82. A polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised by either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 1-10% of the N-terminus and/or 1-10% of C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 1-10% of the N-terminus and/or C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

83. A polypeptide, said polypeptide selected from the group consisting of (1) a polypeptide comprised by SEQ ID NO:1 provided that a portion of SEQ ID NO:1 is missing in the polypeptide, said missing portion selected from the group consisting of a signal peptide, a membrane association sequence, and a cell association sequence, and (2) a polypeptide comprised by SEQ ID NO:2 provided that a portion of SEQ ID NO:2 is missing in the polypeptide, said missing portion selected from the group consisting of a signal peptide, a membrane association sequence, and a cell association sequence.

84. A polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised by either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 5-15% of the N-terminus and/or C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 5-15% of the N-terminus and/or C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

85. A polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised by either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 10-25% of the N-terminus and/or C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 10-25% of the N-terminus and/or C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

86. A polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised by either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 15-45% of the N-terminus and/or C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 15-45% of the N-terminus and/or C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

87. The method of Claim 49, in which said measuring the individual's bodily fluid level of an angiocidin fragment or fragments further comprises the use of an angiocidin fragment as a standard.

88. The method of Claim 87, in which said angiocidin fragment used as a standard is selected from the group consisting of a recombinant angiocidin fragment, a purified angiocidin fragment that occurs in a mixture with other angiocidin fragments, and a partially purified angiocidin fragment.

89. A method to detect the presence and/or clinical course of a disease by assaying a bodily fluid from an individual, wherein the method comprises the steps of:

(1) measuring the individual's bodily fluid level of an angiocidin fragment or fragments; and

(2) utilizing the result of step (1) in a diagnosis as to whether the individual has a disease and/or whether a known disease has progressed, regressed, or remained stable.

ABSTRACT OF THE DISCLOSURE

This invention relates to a method of detecting an angiocidin fragment in the blood, blood plasma, serum, another bodily fluid, a biopsy or other tissue, of a mammal, especially a human, as a means of detecting, diagnosing and/or following a cancer.

APPLICATION DATA SHEET

Electronic Version 0.0.11

Stylesheet Version: 1.0

Attorney Docket Number: W1107/20007

Publication Filing Type:	new-utility
Application Type:	provisional
Title of Invention:	ANGIOCIDIN FRAGMENTS AND USES THEREOF IN CLINICAL ASSAYS FOR CANCER

Customer Number Attorney: 03000

03000

Customer Number Correspondence Address: 03000

03000

INVENTOR(s):

Primary Citizenship:	United States
Given Name:	Kevin
Middle Name:	J.
Family Name:	WILLIAMS
Residence City:	Wynnewood
Residence State:	PA
Residence Country:	US
Address:	425 Wister Road
	Wynnewood PA, 19096 US